



APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

RECEIVED

JUN 28 2002

In the Specification:

At page 1, please amend the paragraph beginning on line 3 as follows: TECH CENTER 1600/2900

RELATED APPLICATIONS

This application is a continuation of U.S. Application No. 09/134,403, filed August 14, 1998, [pending]now issued as U.S. Patent 6,107,093, which is a continuation of U.S. Application No. 08/363,868, filed December 17, 1994, issued as U.S. Patent 5,821,093, which is a continuation-in-part of U.S. Application No. 08/013,658 filed February 4, 1993, now abandoned, which is a continuation of 07/624,227, filed December 7, 1990, now abandoned, which is a continuation-in-part of 07/352,062, filed May 15, 1989, issued as U.S. Patent 5,000,000, which is a continuation-in-part of 07/239,099, filed August 31, 1988, now abandoned, and is a continuation-in-part of 07/946,290, filed September 17, 1992, issued as U.S. Patent 5,487,989.

At Page 14, please amend the paragraph beginning at line 10 and ending at line 27, as follows:

Figures [3A-3F]3A-3G illustrate[s] ethanol (A, C, E, G) production and growth (B, D, F) during batch fermentation. A and B. Fermentation of 10% glucose. Symbols: ●, plasmid-based ethanol production strain ATCC11303(pLOI297); O, chromosomally-integrated strain lacking an expression enhancing mutation, KO2. C. and D. Fermentation of 10% glucose. Symbols: O, a chromosomally- integrated strain lacking a spontaneous expression enhancing mutation, KO3; ●, chromosomally-integrated strain containing a spontaneous expression enhancing mutation, KO4; ▲, KO4 supplemented with 22 mM sodium acetate. E. and F. Fermentation of 8% xylose. Symbols: ●, KO4; ▲, KO11, a further mutant of KO4, lacking fumarate reductase activity (*frd*); □,

A

KO12, a further mutant of KO11, carrying a *recA* mutation. G. Fermentation by KO20, a chromosomally-integrated strain with an induced mutation. Symbols: ■, 10% glucose, ●, 8% xylose.

At Page 14, please amend the paragraph beginning at line 31 and ending at page 15, line 2, as follows:

Figures 5 and 6 illustrate the ethanol production by recombinant strains of *K. oxytoca* M5A1. Figure 5 illustrates the ethanol production from glucose (100 g/liter) by: (●) strain M5A1 (pLOI555; ATCC 68564), (▲) strain P2 containing integrated *PET* genes, and (■) strain B1 containing integrated *PET* genes. Figure 6 illustrates the ethanol production from cellobiose (100 g/liter) fermentation by strain P2 (ATCC 55307). (▲), ethanol; (Δ), cell mass.

At Page 20, please amend the paragraph beginning on line 28 and ending on line 32, as follows:

**Biological deposits:** The following cultures have been deposited with the American Type Culture Collection (ATCC), [12301 Parklawn Drive, Rockville, Maryland, 20852] presently located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. Table 1 lists the accession numbers assigned to the cultures by the repository.

At Page 48, please amend the paragraph beginning on line 19 and ending on page 49, line 15, as follows:

After overnight growth in liquid culture, 0.1 ml of stationary phase cells was spread on plates containing 600 µg Cm/ml and 2% glucose to select for high level expression. Single large colonies were retained from each independent integration event and named according to the restriction site used for construction, i.e., S1, S2, S3, P1, P2,

and B1. These colonies were tested for the presence of plasmids by transformation of miniscreen DNA and for expression of *PET* genes on aldehyde indicator plates (Table 5). Putative integrated strains found to contain plasmid-borne *cat* genes were discarded after digesting miniscreen DNA to confirm the presence of pLOI510 (presumably a low level contaminant of gel-purified fragments). The parent organism and M5A1 (pLOI555; ATCC 68564), an excellent ethanol producer, were included as negative and positive controls, respectively. Two clones expressed the ethanol genes at levels nearly equivalent to that of M5A1 (pLOI555), strains B1 and P2 (ATCC 55307). Plasmid LOI510, containing *PET* genes and *cat* within the *E. coli pfl* gene was digested with *Sall*, or *PstI*, circularized and transformed into *K. oxytoca* strain M501. See Wood *et al.*, *Appl. Environ. Microbiol.* 58:2103 (1992), which is herein incorporated by reference. Additionally, homologous *K. oxytoca* M5A1 DNA prepared by *Sau3A* digestion was ligated to a 4.6 kb *Bam*H1 fragment containing only the *PET* and *cat* genes was also used for transformation. Recombinants were initially selected using 20 µg chloramphenicol/ml and expressed low levels of *Z. mobilis* enzymes. As with *E. coli* (Ohta *et al.* [1991] *Appl. Environ. Microbiol.* 57: 893-900), expression was boosted by direct selection of mutants with resistance to 600 µg chloramphenicol/ml. A single clone expressing high level resistance was retained for each independent integration event.

**In the Claims:**

Please cancel without prejudice or disclaimer claims 6, 17-24, 25-34, 40, 42-45 and 47.

Please amend claims 48 and 49 as follows:

23/48. (Amended) The recombinant host cell strain[,] according to claim 1, of *Klebsiella oxytoca* [P2]M5A1 comprising a plasmid represented by a deposit with the American Type Culture Collection designated as deposit number ATCC 68564.

29 49. (Amended) A cell strain according to claim <sup>70</sup>11, wherein said cell strain is  
designated P2 and represented by a deposit with the American Type Culture Collection  
designated as deposit number ATCC [number]55307.